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[Suite sur la page suivante]

(54) Title: NOVEL METHOD FOR ANALYZING NUCLEIC ACID AND USE THEREOF FOR EVALUATING THE DEGREE OF MRNA EDITING OF THE SEROTONIN 5-HT$_{2C}$ RECEPTOR

(54) Titre : METHODE D'ANALYSE D'ACIDES NUCLEIQUES ET SON UTILISATION POUR EVALUER LE DEGRE D'EDITION DE L'ARNM DU RECEPTEUR 5-HT$_{2C}$ DE LA SEROTONINE

(57) Abstract: The invention concerns a method for analyzing nucleic acids using a small-size probe array comprising deoxyinosines (dI) instead of deoxyguanosines (dG). The invention also concerns such probe arrays and their use in methods for detecting and/or quantifying target oligonucleotides present in DNA (deoxyribonucleic acid) or RNA (ribonucleic acid) molecules in a sample, in particular mRNA editing rate of the serotonin 5-HT$_{2C}$ receptor (5-HT$_{2C}$-R). The invention further concerns a biochip or a reactor in liquid medium comprising such probe arrays as well as their uses, in particular for detecting and/or identifying genetic polymorphisms or for determining an mRNA editing rate, whether it is that of a 5-HT$_{2C}$-R mRNA or any other RNA capable of being edited. The invention also concerns a method based on the isolation of a single strand conformation polymorphism (SSCP) enabling under specific analysis conditions the editing profile and/or rate of an mRNA capable of being edited to be obtained, as well as a method for diagnosing diseases or susceptibility to diseases associated with the degree of edition of an mRNA. Finally, the invention concerns a method for selecting compounds capable of modulating mRNA editing rate, in particular that of 5-HT$_{2C}$-R, as well as the use of such compounds for preparing a pharmaceutical composition for treating organic fluid.

(57) Abrégé : La présente invention se rapporte à une méthode d'analyse des acides nucléiques mettant en oeuvre un jeu de sondes de petite taille incluant des désoxyinosines (dI) à la place de désoxyguanosines (dG). L'invention comprend également de tels jeux de sondes ainsi que leur utilisation dans des procédés de détection et/ou de quantification d'oligonucléotides cibles présents dans des molécules d'ADN (acide désoxyribonucléique) ou d'ARN (acide ribonucléique) dans un échantillon, notamment la détermination du taux d'édition de l'ARN messager (ARNm) du récepteur 5-HT$_{2c}$ (5-HT$_{2c}$-R) de la sérotonine. L'invention est aussi relative à une biopuce ou à un réacteur en milieu liquide comprenant de tels jeux de sondes ainsi que leurs utilisations, notamment pour la détection et/ou l'identification de polymorphismes génétiques ou pour la détermination du taux d'édition d'un ARNm, que ce soit celui de l'ARNm du 5-HT$_{2c}$-R ou de tout autre ARN susceptible d'être édité. La présente invention a également pour objet une méthode fondée sur la mise en évidence de polymorphisme de conformation de l'ADN simple brin (SSCP) permettant dans des conditions données d'analyse d'obtenir le profil et/ou le taux d'édition d'un ARNm susceptible d'être édité, ainsi qu'une méthode de diagnostic de maladies ou de susceptibilité à des maladies associées au degré d'édition d'un ARNm. La présente invention a aussi pour objet une méthode de sélection de composés capables de moduler le taux d'édition de l'ARNm, notamment celui du 5-HT$_{2c}$ R, ainsi que l'utilisation de tels composés pour la préparation d'une composition pharmaceutique destinée au traitement de l'humeur.

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FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), brevet OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Déclaration en vertu de la règle 4.17 :

— *relative à la qualité d'inventeur (règle 4.17.iv)) pour US seulement*

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En ce qui concerne les codes à deux lettres et autres abréviations, se référer aux "Notes explicatives relatives aux codes et abréviations" figurant au début de chaque numéro ordinaire de la Gazette du PCT.

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PCT/FR2003/002339

Translation

PATENT COOPERATION TREATY

PCT

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 345098 D20534	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FR2003/002339	International filing date (*day/month/year*) 24 juillet 2003 (24.07.2003)	Priority date (*day/month/year*) 26 juillet 2002 (26.07.2002)
International Patent Classification (IPC) or national classification and IPC C12Q 1/68, C12Q1		
Applicant BIOCORTECH		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 26 février 2004 (26.02.2004)	Date of completion of this report 24 November 2004 (24.11.2004)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

ational application No.
PCT/FR2003/002339

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-49, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the claims:
pages 1-39, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the drawings:
pages 1/1, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/FR2003/002339

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☐ all parts.
- ☒ the parts relating to claims Nos. _____

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/[redacted]03/02339

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	28-37 (in full), 38 and 39 (partially)	YES
	Claims		NO
Inventive step (IS)	Claims		YES
	Claims	28-37 (in full), 38 and 39 (partially)	NO
Industrial applicability (IA)	Claims	28-37 (in full), 38 and 39 (partially)	YES
	Claims		NO

2. Citations and explanations

This report refers to the following documents:

D1: FUCHS MELANIE ET AL: "RNA editing in higher plant plastids: Oligoribonucleotide SSCP analysis allows the proof of base conversion directly at the RNA level" CURRENT GENETICS, vol. 39, no. 5-6, July 2001 (2001-07), pages 384 to 387.

D2: ZHONG SHAOBIN ET AL: "Detection of apolipoprotein B mRNA editing by peptide nucleic acid mediated PCR clamping" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 259, no. 2, 7 June 1999 (1999-06-07), pages 311 to 313.

D3: NISWENDER COLLEEN M: "Strategies and requirements for the detection of RNA editing in G protein coupled-receptor RNA." METHODS IN ENZYMOLOGY. UNITED STATES 2002, vol. 343, 2002, pages 476-492.

D4: MAEKAWA MASATO ET AL: "Relative ratios of mRNA molecules encoded by genes with homologous sequences using fluorescence-based single-strand conformation polymorphism analysis" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 223, no. 3, 1996, pages 520-525.

D5: IBRAHIM A F ET AL: "Differential expression of potato U1A spliceosomal protein genes: a rapid method for

expression profiling of multigene families." PLANT MOLECULAR BIOLOGY. MAR 2001, vol. 45, no. 4, March 2001 (2001-03), pages 449-460.

D6: ELLISON JANE S: "Fluorescence-based mutation detection: Single-strand conformation polymorphism analysis (F-SSCP)" MOLECULAR BIOTECHNOLOGY, vol. 5, no. 1, 1996, pages 17 to 31.

D7: WO 02/38809 A (INGENY HOLDING BV; VOS GERRIT JOHANNIS DE (NL)) 16 May 2002 (2002-05-16).

1. NOVELTY (PCT Article 33(2))

1.1 Among the documents cited in the international search report, D4 has the most technical features in common with the subject matter of claim 28. Said document describes an SSCP method for analysing mRNA that differs from the method according to claim 28 in that the separation of single-strand DNA is performed by electrophoresis on gel (see "SSCP analysis", pages 521 to 522) and not by capillary electrophoresis.

1.2 Within the meaning of PCT Article 33(2), the subject matter of claims 28 to 37 (completely), 38 and 39 (partially) is novel over the prior art cited in the international search report.

2. INVENTIVE STEP (PCT Article 33(3))

2.1 D1, which is considered to be the prior art closest to the subject matter of claims 28 and 33, describes an SSCP method enabling the profile and the editing rate of an editable RNA to be obtained from a sample of eucaryote cells (page 385, column 1, "Plant growth..."), including the steps of extracting all the

chloroplastic RNA from the sample; RNase T1 digestion; fractioning by ion exchange chromatography then rSSCP; and detection by hybridization using a radioactive probe (page 385, column 2 to page 386, column 1 and figure 1).

2.2 The subject matter of claims 28 and 33 differs from D1 in that the method used includes a step of RT-PCR amplification prior to SSCP detection, with separation of the single-strand DNA by capillary electrophoresis and fluorescence detection.

2.3 The problem that the present invention is intended to solve can therefore be considered to be that of providing an improved SSCP method for obtaining with greater sensitivity the editing profile (claim 28), or the editing profile and the editing rate (claim 33) of an editable mRNA.

2.4 The proposed solution consists in using an RT-PCR amplification method prior to SSCP detection with separation of the single-strand DNA by capillary electrophoresis and fluorescence detection.

2.5 However, this solution cannot be considered to involve an inventive step for the following reasons:

2.5.1 Claim 28 relates to an "SSCP method [...] for obtaining the editing profile of an *editable* mRNA" (emphasis added). The mRNA analysed by the method of claim 28 is therefore not necessarily effectively edited. Furthermore, in the light of the definition of editing given on page 10 of the description of the present application (deamination of adenosines to inosines) and the far more general definition

given in D3 (page 476, introduction, lines 1 to 3: any RNA-modifying event (excluding splicing) resulting in a messenger, the sequence of which differs from the corresponding genomic DNA), it is clear that determining the editing profile of an mRNA is a special case of determining sequence variations present therein.

2.5.2 D4 describes an SSCP method for determining sequence variations present in the mRNA, in particular two homologous sequences encoding the subunits A and B of lactate deshydrogenase, including the steps of extracting all the RNA from the sample; **reverse transcription of the extracted RNA and synthesis of the double-strand cDNA** (page 521, "RNA preparation and cDNA synthesis"); PCR amplification of the cDNA by means of a pair of primers specific to said mRNA, said pair of primers being chosen to enable all forms of said mRNA having sequence variations to be amplified and said primers being labelled by fluorophores (page 521, "Design and synthesis of fluorescence-labeled primers", "Procedure of PCR", and figure 1); dissociation of the double-strand DNA into single-strand DNA (page 522, line 3); separation of the single-strand DNA by electrophoresis; and obtaining the sequence variation profile by **reading the fluorescence** (page 522, lines 4 to 7; figure 2).

2.5.3 D5 describes an SSCP method for determining sequence variations present in the mRNA, in particular two fragments differing by two point mutations (figure 4) originating from genes of the U1A family, including the steps of extracting all the RNA from the samples (page 450, column 2, "Nucleic acid

extraction..."); **reverse transcription of the RNA; synthesis of the double-strand cDNA;** and PCR amplification by means of a pair of primers specific to said mRNA enabling the various forms of the mRNA potentially present to be amplified (page 451, column 1, "RT-PCR..."; figure 4); dissociation of the double-strand DNA to single-strand DNA (implicit in any SSCP method); separation of the single-strand DNA by electrophoresis on gel; and acquisition of the profile (page 451, column 1, "Single-stranded conformational polymorphism (SSCP)"; figure 4).

2.5.4 In the light of documents D4 or D5, it is clear that the RT-PCR method combined with SSCP analysis is well known in the art of detecting sequence variations between mRNA molecules of related sequences.

2.5.5 When addressing the problem to be solved, as defined in point 2.3 above, a person skilled in the art would consider it obvious to modify the method according to D1 by adding an RT-PCR step prior to SSCP detection according to D4 or D5 and fluorescence detection according to D4, with a view to increasing the sensitivity with which the sequence variations of an editable mRNA can be detected.

2.5.6 The fact that the detection step is performed by capillary electrophoresis in claims 28 and 33 instead of electrophoresis on gel in the methods described in D4 and D5 is not considered to involve an inventive step, since both methods are well known equivalents to a person skilled in the art (for an illustration of electrophoresis on gel, see D6; for

capillary electrophoresis, see D7).

2.5.7 Dependent claims 29 to 32 contain no additional feature which, when combined with the features of any one of the claims to which they refer, defines subject matter that complies with the PCT requirements of inventive step, for the following reasons: editing sites of the 5-HT2c receptor are well known (see D3, figure 2) and therefore constitute a set of options among a number of other possibilities for applying the SSCP detection method.

2.5.8 Independent claims 34 to 39 do not involve an inventive step either, since they relate to customary uses in the art (i.e. for selecting compounds or diagnosing) of a detection method that does not in itself involve an inventive step.

2.6 In the light of the above, the present application fails to meet the requirements of PCT Article 33(1), since the subject matter of claims 28 to 37 (in full), 38 and 39 (partially) does not involve an inventive step.

3 INDUSTRIAL APPLICABILITY (PCT Article 33(4))

3.1 The subject matter of claims 28 to 39 is industrially applicable within the meaning of PCT Article 33(4).

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV

1. The International Searching Authority agrees with the objection raised by the International Preliminary Examining Authority with regard to the lack of unity of invention; the arguments put forward in the annex to the partial international search report (invitation to pay additional fees; form PCT/ISA/206) are upheld in their entirety (see also the PCT International Search Guidelines and International Preliminary Examination Guidelines, 10.71 to 10.77). Said arguments are restated hereunder.

1.1 The present application fails to meet the requirements of PCT Rule 13.1, since it relates to multiple inventions that are not so mutually linked as to form a single general inventive concept.

1.2 Inventions 1 and 2 are linked by the common concept of providing products or methods for detecting and/or quantifying edited or non-edited forms of RNA.

1.3 However, products or methods for detecting and/or quantifying edited or non-edited forms of RNA are known from the prior art. For example, D1 (Fuchs et al., Current Genetics (2001) 39: 384 to 387) describes detecting editing events by conversion using the rSSCP method; D2 (Zhong et al., Biochem. Biophys. Res. Comm. (1999) 259: 311 to 313) describes a method for detecting RNA editing including the use of a PNA ("peptide nucleic acid") enabling the PCR amplification of the non-edited

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV

RNA to be blocked and the edited RNA to be detected selectively; and D3 (Niswender et al., Meth. Enzymol. (2002) 343: 476 to 492) describes detecting RNA editing events of the human serotonin 5-HT2c receptor by inserting sites of specific restrictions of the edited or non-edited forms, primer extension, or RT-PCR followed by sequencing.

1.4 In the light of the above prior art, the concept common to inventions 1 and 2 is not novel, and the problem solved by the present application can be restated as that of providing alternative products or methods for detecting and/or quantifying edited or non-edited forms of mRNA.

1.5 The proposed solutions to this problem consist in providing, on the one hand, sets of oligodeoxyribonucleotide probes wherein at least one dG nucleotide on at least one of said probes has been substituted by a dI nucleotide, products and methods using said sets of probes (invention 1) and, on the other hand, single-strand conformation polymorphism-based (SSCP) detection methods (invention 2).

1.6 Since the proposed solutions to the problem cannot be linked by an inventive feature or special technical relationship and since the International Searching Authority was unable to identify any other special technical feature, within the meaning of PCT Rule 13.2, common to all the inventions, it is not possible to define a general inventive

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV

concept covering inventions 1 and 2.

1.7 Consequently, the present application lacks unity of invention and fails to comply with the criteria of PCT Rule 13.1. The different inventions are therefore stated as follows:

1.7.1 Invention 1: claims 1 to 27 (in full), 38 and 39 (partially); set of at least two different oligodeoxyribonucleotide probes for detecting and/or quantifying an oligonucleotide derived from a fragment of mRNA coding for a mammalian cell membrane receptor, wherein at least one dG nucleotide on at least one of said probes has been substituted by a dI nucleotide, biochip, reactor, device and kit including said set of at least two probes, the use of said biochip or said device, methods for detecting and/or quantifying oligonucleotides or for determining the percentage of each of the edited or non-edited forms of an mRNA in a sample using said biochip or said device, and methods for selecting compounds using said method to detect and/or quantify oligonucleotides.

1.7.2 Invention 2: claims 28 to 37 (in full), 38 and 39 (partially); SSCP methods for obtaining the editing rate and/or profile of an mRNA, methods for selecting compounds or diagnostic methods using said SSCP methods.

1.8 Inventions 1 and 2 were the subject of an international search report.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: IV

2. In accordance with the applicant's request to have the international search, for which a single examination fee has been paid, performed on the second group of inventions (claims 28 to 37 (in full), 38 and 39 (partially)), this report has been limited to invention 2 as indicated above.